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Rapid monitoring of iron-chelating therapy in thalassemia major by a new cardiovascular MR measure: the reduced transverse relaxation rate

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Abstract

In iron overload, almost all the excess iron is stored intracellularly as rapidly mobilizable ferritin iron and slowly exchangeable hemosiderin iron. Increases in cytosolic iron may produce oxidative damage that ultimately results in cardiomyocyte dysfunction. Because intracellular ferritin iron is evidently in equilibrium with the low-molecular-weight cytosolic iron pool, measurements of ferritin iron potentially provide a clinically useful indicator of changes in cytosolic iron. The cardiovascular magnetic resonance (CMR) index of cardiac iron used clinically, the effective transverse relaxation rate (R_2^*), is principally influenced by hemosiderin iron and changes only slowly over several months, even with intensive iron-chelating therapy. Another conventional CMR index of cardiac iron, the transverse relaxation rate (R_2), is sensitive to both hemosiderin iron and ferritin iron. We have developed a new MRI measure, the ‘reduced transverse relaxation rate’ (RR_2), and have proposed in previous studies that this measure is primarily sensitive to ferritin iron and largely independent of hemosiderin iron in phantoms mimicking ferritin iron and human liver explants. We hypothesized that RR_2 could detect changes produced by 1 week of iron-chelating therapy in patients with transfusion-dependent thalassemia. We imaged 10 patients with thalassemia major at 1.5 T in mid-ventricular short-axis planes of the heart, initially after suspending iron-chelating therapy for 1 week and subsequently after resuming oral deferasirox. After resuming iron-chelating therapy, significant decreases were observed in the mean myocardial RR_2 (7.8%, $p < 0.01$) and R_2 (5.5%, $p < 0.05$), but not in R_2^* (1.7%, $p > 0.90$).

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Although the difference between changes in RR_2 and R_2 was not significant ($p > 0.3$), RR_2 was consistently more sensitive than R_2 (and R_2^*) to the resumption of iron-chelating therapy, as judged by the effect sizes of relaxation rate differences detected. Although further studies are needed, myocardial RR_2 may be a promising investigational method for the rapid assessment of the effects of iron-chelating therapy in the heart.

Keywords

MRI; heart; cardiomyopathy; iron chelation; R_2

INTRODUCTION

Iron-induced cardiomyopathy remains the most common cause of death in patients with transfusion-dependent thalassemia (1), despite encouraging progress in diagnosis and management (2). Recently published evidence indicates that the use of cardiovascular magnetic resonance (CMR) measurement of the effective transverse relaxation rate (R_2^*) ($= 1/T_2^*$) as a screening tool can identify patients with thalassemia major who are at high risk for the development of cardiac failure and arrhythmias, and who could benefit from intensified iron-chelating therapy (3). Nonetheless, improved means for the rapid monitoring of the effectiveness of an iron-chelating regimen in clearing iron from the heart are still needed. Myocardial R_2^* changes only slowly over several months, even with intensive continuous iron-chelating therapy with parenteral deferoxamine, despite rapid improvement in cardiac function (4). A recent trial of the oral iron chelator deferasirox in patients with cardiac iron deposition found no significant difference in R_2^* after 6 months; 1 year of observation was needed to detect a significant change (5).

The sluggish response of myocardial R_2^* to iron chelation is a consequence of the differential sensitivity of this relaxation rate to the various forms of iron within the cardiomyocyte. In transfusional iron overload, almost all the excess iron is sequestered intracellularly as ferritin iron, a soluble and rapidly mobilizable fraction dispersed within the cytoplasm, and hemosiderin iron, an insoluble aggregate within lysosomes that serves as a long-term reserve. Hemosiderin iron is considered to be derived from ferritin iron within specialized lysosomes (siderosomes) for prolonged storage (6). The exact species of iron that produce toxicity are uncertain (7), but a number of studies have identified the very small, micromolar concentrations of metabolically active low-molecular-weight iron in the cytosol as responsible (7–9). Increases in cytosolic iron may produce oxidative damage that ultimately results in cardiomyocyte dysfunction and death, cardiac injury and failure (10–13). No noninvasive methods permit a direct measurement of the cytosolic low-molecular-weight iron pool in the heart. Because intracellular ferritin iron is evidently in equilibrium with the low-molecular-weight cytosolic iron pool (14), measurements of ferritin iron potentially provide a clinically useful indicator of changes in cytosolic iron. Myocardial R_2^* is most sensitive to lysosomal hemosiderin (2) and is little affected by either cytoplasmic low-molecular-weight iron or ferritin. Another conventional CMR index of cardiac iron, the transverse relaxation rate R_2 , is sensitive to both hemosiderin iron and ferritin iron. We have developed a new relaxation rate, the ‘reduced transverse relaxation rate’ (RR_2), and have proposed in previous studies (15,16) that this measure is primarily sensitive to ferritin iron and largely independent of hemosiderin iron. In addition, our method quantifies hemosiderin iron separately, with the intent of improving the accuracy of determinations of total (hemosiderin plus ferritin) storage iron (16). Earlier studies have provided initial evidence for the ability of our method to separately estimate ferritin iron and hemosiderin iron in both surrogate phantoms mimicking the two forms of iron and in human tissue (16,17).

We hypothesized that the new CMR relaxation rate RR_2 may provide a new noninvasive means of rapidly monitoring the effects of iron-chelating therapy in the heart. The purpose of our study was to compare the sensitivity of RR_2 (primarily sensitive to ferritin iron) with R_2 (sensitive to both ferritin and hemosiderin iron) and R_2^* (primarily sensitive to hemosiderin iron) in detecting changes produced by 1 week of iron-chelating therapy in patients with transfusion-dependent thalassemia who had previously suspended chelation for 1 week.

METHODS

Patient population

Ten patients with transfusion-dependent thalassemia (seven males; three females; mean age, 26.9 ± 10.3 years) were imaged using a 1.5-T whole-body MR scanner. Patients were over 10 years of age, on regular red cell transfusions, and all were on stable chelation regimens with deferasirox at 20–30 mg/kg/day. Human imaging was performed in accordance with protocols approved by the New York University School of Medicine Institutional Review Board; all subjects provided written informed consent or assent (if under 18 years of age, with written consent from parent or guardian). Each patient was imaged in a mid-ventricular short-axis plane of the heart, initially after suspending iron chelation for 1 week (Day 7) and, secondly, after resuming their usual therapy for 1 week (Day 14). Anatomical landmarks were used to reproduce the same imaging planes between time points.

Pulse sequence

We used the previously proposed breath-hold multi-echo fast spin-echo (ME-FSE) pulse sequence (18) that has been shown to yield good image quality and accurate R_2 in controls and patients with thalassemia. Both the breath-hold ME-FSE (18) and R_2^* (19) pulse sequences were implemented on a 1.5-T whole-body MR scanner (Avanto, Siemens Healthcare, Erlangen, Germany) equipped with a gradient system capable of achieving a maximum gradient strength of 45 mT/m and a slew rate of 200 T/m/s. The radiofrequency (RF) excitation was performed using the transmit body coil, and a 32-element cardiac coil array (Invivo, Orlando, FL, USA) was employed for signal reception. Relevant imaging parameters for the ME-FSE pulse sequence included the following: field of view, 340×276 mm²; matrix, 128×72 ; spatial resolution, 2.7×3.8 mm²; slice thickness, 10 mm; generalized autocalibrating partially parallel acquisitions (20) with an effective acceleration factor of 1.6; receiver bandwidth (BW), 500 Hz/pixel; excitation RF pulse duration, 2.1 ms; refocusing RF pulse duration, 2.6 ms; echo spacing (ESP), 5.6 ms; number of images, 10; echo-train duration, 118 ms; repetition time, 1 heart beat; breath-hold duration, 22 heart beats; double inversion recovery, black-blood preparation pulse. The second ME-FSE acquisition was performed with identical imaging parameters except for ESP = 7 ms, BW = 295 Hz/pixel and number of images = 8. The third ME-FSE acquisition was performed with identical imaging parameters except ESP = 10 ms, BW = 155 Hz/pixel and number of images = 6. The presence of aggregated, insoluble hemosiderin iron produces non-monoexponential ME-FSE signal decay with a strong dependence on ESP because of water diffusion effects (15,16). We acquired three different ME-FSE acquisitions with different ESPs to calculate RR_2 . Having multiple ESP data helps to constrain the parameters of our fitting model, which predicts a specific ESP dependence for the water diffusion effects associated with hemosiderin. To minimize stimulated echoes from imaging slice edges, the slice thickness of the refocusing RF pulse was set to three times that of the excitation RF pulse (18,21). The relevant R_2^* pulse sequence parameters were as follows: spatial resolution, 2.7×3.8 mm²; slice thickness, 10 mm; double inversion recovery, black-blood preparation pulse; BW = 1500 Hz/pixel; first echo time (TE), 1.9 ms; $\Delta TE = 0.97$ ms; number of images, 12; breath-hold duration, 10 heart beats.

Image analysis

R_2^* was calculated by nonlinear least-squares fitting for three parameters of the monoexponential signal relaxation equation:

$$S(t) = (S_{\text{ideal}}^2 + \sigma^2)^{1/2}; S_{\text{ideal}} \equiv S_0 e^{-R_2^* t} \quad [1]$$

where $S(t)$ is the signal amplitude at time t , S_{ideal} is the ideal (or unbiased) signal, and the three unknown parameters are the initial signal amplitude (S_0), the mean background noise (σ) and R_2^* . This approximate noise correction procedure is similar to the method of McGibney and Smith (22), although here the noise was determined from a fit to the data rather than from the signal in air. Similarly, R_2 was calculated for only the ME-FSE dataset with the shortest ESP using an equation of the same form (eqn [1]). R_2 was not calculated for the ME-FSE datasets with longer ESPs, because they were more influenced by the magnetic field inhomogeneities and water diffusion effects associated with hemosiderin iron than were the shortest ESP data.

As has been proposed previously (15,16), the non-monoexponential signal decay in iron-overloaded tissue can be approximately modeled as:

$$S_{\text{ideal}}(t) = S_0 e^{-RR_2 t} \exp \left[-A^{3/4} (\Delta t)^{3/4} (t)^{3/8} \right] \quad [2]$$

where $2\Delta t$ is the inter-ESP. The three unknown parameters are S_0 , RR_2 and the aggregation index (A). In addition, a noise parameter was included in the manner suggested by eqn [1], resulting in a total of four fitting parameters for this model. As our model gives a specific ESP dependence, a global fit for the full dataset with three different ESP values was applied, thereby strongly constraining the model parameters. Our nonlinear Levenberg–Marquardt fitting routine generally converged quickly to a unique solution. The initial estimate for S_0 was made as twice the signal of the first TE image for ESP = 5.6 ms. The remaining three initial estimates were based on prior knowledge of preliminary data. Different initial estimates were used to test the robustness of the fitting procedure and, in the few cases in which more than one solution was found, the solution that minimized the mean square residuals was selected.

Image analysis was performed using customized analysis software developed in MATLAB® (R2008a software; Mathworks, Natick, MA, USA). Nonlinear least-squares fitting was based on the MATLAB implementation of the Levenberg–Marquardt algorithm (Statistics Toolbox™ version 6.2). For relaxation rate quantification, myocardial contours were manually segmented with care to avoid partial volume effects. To minimize measurement errors (e.g. R_2^*) caused by non-iron-related static magnetic field inhomogeneities, only the septum was used to calculate the relaxation rates (23).

We used two approaches to calculate the relaxation rates. One approach was to first average the signal within the region of interest (ROI) and then perform data fitting to calculate the relaxation rate for the ROI. The second approach was to first perform pixel-by-pixel data fitting and then perform averaging of the relaxation rate within the ROI. The pixel-by-pixel approach was used for visual assessment, but only the ROI approach was included for statistical analysis.

The analysis of three different ME-FSE datasets acquired with different breath-holds required image registration. We implemented a Fourier-based algorithm based on rigid body transformation to co-register the three different ME-FSE datasets, and this image-to-image

algorithm has been shown to produce subpixel accuracy under the condition of rigid body transformation (24). For convenience, the second and third ME-FSE datasets were registered to the first ME-FSE dataset. After performing image registration, a single set of myocardial contours was used for all three ME-FSE datasets with different ESPs, and RR_2 was calculated using both the pixel-by-pixel and ROI approaches.

Statistical analysis

The patient datasets were pooled and randomized for blinded analysis. Intra- and inter-observer variabilities in R_2^* , R_2 and RR_2 measurements were assessed by performing Bland–Altman analysis. The intra-observer variability of one blinded observer was assessed by repeating the image analysis from the same set of images with at least 2 weeks of separation from the first analysis. The second blinded observer performed the image analysis independently. Both observers were blinded to the subject identity and study time point.

Statistical analyses were performed using Analyse-it for Microsoft Excel version 2.20 (Analyse-it Software, Ltd., Leeds, UK). The mean relaxation rates were compared between measurements obtained 1 week after suspending chelation and those obtained 1 week after resuming chelation, using a paired t -test (two-tailed). $p < 0.05$ was considered to be statistically significant. For each relaxation rate, the mean absolute difference was calculated between the two chelation states for each group. In addition, the corresponding mean percentage difference was calculated by normalizing the absolute difference by the mean of the two states, and then multiplying by 100%. The reported relaxation rates represent the mean $\pm$ standard deviation of observer 1 and analysis 1. To assess the ability of the various relaxation rates to detect changes produced by the 1-week suspension or resumption of iron-chelating therapy, we calculated the magnitude of the effect size of the observed change in relaxation rates using Cohen's d statistic (25).

RESULTS

Five of 10 patients ($T_2^* < 20$ ms) had clinical evidence of cardiac iron overload (26). Figure 1 shows representative ME-FSE images before and after performing image registration. It should be noted that the third ME-FSE image with ESP = 10 ms (third column in Fig. 1) was originally shifted with respect to the first ME-FSE image, and was adequately co-registered with the other two ME-FSE images after using the registration algorithm. Figure 2 shows representative log signal vs time curves for the three ESPs used in this study, as well as the corresponding signal fits. The individual R_2 values calculated using the monoexponential equation were 20.6, 23.8 and 26.8 ms for ESPs of 5.6, 7 and 10 ms, respectively, whereas the corresponding RR_2 value calculated using the non-monoexponential equation was 17.4 ms. These findings are consistent with the theory of non-monoexponential signal decay in iron overload (15,16). Figure 3 shows representative pixel-by-pixel R_2^* , R_2 and RR_2 maps of a patient after 1 week off and, thereafter, 1 week on iron chelation.

Between day 7 and Day 14, the mean RR_2 and R_2 values decreased significantly with the resumption of iron-chelating therapy (see Table 1 for details). By contrast, R_2^* did not change significantly after restarting iron-chelating therapy. The mean absolute (percentage) decrease (Day 14 – Day 7) in RR_2 was $-1.7 \pm 1.3 \text{ s}^{-1}$ ($-7.8 \pm 6.2\%$; $p < 0.01$), and eight of 10 patients showed a decrease in RR_2 (Fig. 4). The mean absolute (percentage) decrease in R_2 was $-1.5 \pm 1.5 \text{ s}^{-1}$ ($-5.5 \pm 5.2\%$; $p < 0.05$), and 10 of 10 patients showed a decrease in R_2 (Fig. 4). The mean absolute (percentage) decrease in R_2^* was $0.5 \pm 11.5 \text{ s}^{-1}$ ($-1.7 \pm 27.4\%$; $p > 0.9$), and three of 10 patients showed a decrease in R_2^* (Fig. 4). Compared with R_2^* and R_2 , RR_2 measured a greater decrease of approximately 8% with the resumption of iron-chelating therapy. The magnitude of the effect size of the change detected using RR_2

was 0.40, which is greater than the effect sizes of the changes detected using R_2 (0.30) and R_2^* (0.02).

As summarized in Table 2, the intra- and inter-observer agreements for RR_2 and R_2 calculations were excellent, whereas the agreement for R_2^* calculation was poorer. Compared with the calculations for the spin-echo-based RR_2 and R_2 , the calculation for the gradient-echo-based R_2^* was more sensitive to manual segmentation of the left ventricular contours. This finding is consistent with a previous study which showed that R_2^* is sensitive to non-iron-related static magnetic field inhomogeneities (27). These results suggest that repeated calculations of RR_2 and R_2 from a given set of images are highly repeatable and reproducible.

DISCUSSION

These results demonstrate the feasibility of detecting changes in the new ‘reduced relaxation rate’ (RR_2) after as little as 1 week of iron-chelating therapy. RR_2 was more sensitive than R_2^* and R_2 to changes produced by resumption of iron-chelating therapy, as judged by the effect sizes of the relaxation rate differences detected. The proposed new CMR method can theoretically distinguish between hemosiderin and ferritin iron *in vivo* (16), potentially permitting the monitoring of changes in both forms of storage iron. As anticipated, myocardial R_2^* , predominantly influenced by hemosiderin iron, did not change significantly with 1 week of iron-chelating therapy. Myocardial R_2 , reflecting both hemosiderin and ferritin iron, decreased significantly over the period of observation, but did not distinguish between the two forms of storage iron. Both R_2 and R_2^* can provide estimates of total tissue iron (28), but cannot be expected to accurately measure ferritin iron concentrations, especially in tissues with severe iron loading, where ferritin iron may represent only a small fraction of the total (16). The intra- and inter-observer agreements for RR_2 calculations from the same set of images were excellent (Table 2).

Our results demonstrate that the proposed CMR method may be a promising investigational technique for the rapid assessment of the effects of iron-chelating therapy in the heart. Nonetheless, these initial studies have limitations that warrant discussion. First, the examinations were carried out over a short time interval in a small number of subjects with a restricted range of iron loading. To fully evaluate the clinical robustness and utility of RR_2 , further studies over longer periods of observation in larger numbers of patients over the whole spectrum of iron overload encountered in clinical practice are required. Such studies would also help to establish the intra- and inter-instrumental, intra- and inter-study, and intra- and inter-observer variability of the technique. Second, the breath-hold duration of 22 heart beats for ME-FSE can be relatively long for some patients with limited breath-hold capacity. Further improvements in data acquisition may be achievable by the use of accelerating techniques, such as k - t sensitivity encoding (SENSE) (29,30) and compressed sensing (31,32), and these methods could be used for patients with limited breath-hold capacity. Third, for the spatial resolution used in this study, the relaxation rate measurements may be sensitive to partial volume effects. This is particularly true for our data as relaxation rate measurements were calculated on the basis of the assumption of robust cardiac image registration, both within each ESP dataset and between different ESP datasets. Within each ESP dataset, gradual ventricular relaxation occurs during the 118 ms of data acquisition in mid-diastole, even with a perfectly still breath-hold. Between different ESP datasets, both ventricular relaxation and breath-hold positions can contribute to registration errors. To minimize registration errors, both within each ESP dataset and between different ESP datasets, we used a thin ROI within the septum. More complex image registration methods are needed to eliminate this potential source of error in data fitting. The aforementioned acceleration techniques may also be used to increase the spatial resolution.

Fourth, although we have provided initial evidence that RR_2 is principally sensitive to ferritin iron in surrogate phantom studies (16) and in human liver explants (17), additional studies in human hearts with iron overload are still needed. More generally, the physiology of myocardial ferritin iron during iron loading and with iron-chelating therapy requires more detailed characterization.

Despite these limitations, RR_2 measurement could be clinically useful in the management of transfusional iron overload in patients with thalassemia major. Compelling evidence indicates that both iron release and incorporation into ferritin are intrinsic, autonomous properties of the molecule (14). Iron entry and exit from ferritin are the result of an equilibrium determined by the concentration of cytosolic iron (14). In human cells, poly(rC)-binding protein 1 (PCBP1) acts as a cytosolic iron chaperone, directly binding and delivering iron to ferritin (33). Confirmation in studies of cardiomyocytes is needed but, together, these observations suggest that myocardial ferritin iron could serve as an indicator of the potentially toxic cytosolic iron pool. Increases in myocardial ferritin iron concentrations may be a useful indicator of increases in cytosolic iron levels, potentially providing an early warning of a heightened risk of iron-induced toxicity.

In future studies, decreases in myocardial RR_2 could offer a means of rapidly monitoring the results of the start or alteration of iron-chelating therapy in patients with transfusion-dependent thalassemia. Recent studies have shown that deferiasirox and deferiprone, the orally active, membrane-permeable iron chelators now in clinical use, lower cytosolic iron, bringing about the release of iron from ferritin, with the iron-depleted ferritin then being monoubiquitinated and digested by the proteasome (34). The net consequence is a reduction in cellular ferritin iron. Deferoxamine, the poorly membrane-permeable iron chelator in use for more than 40 years, decreases cellular ferritin iron by a different route. Deferoxamine enters cells by endocytosis, is localized to lysosomes (35) and induces autophagy of ferritin with digestion of the ferritin within the lysosome (34). Thus, all the iron-chelating agents now used clinically decrease cellular ferritin iron. At present, the response to specific regimens of iron-chelating therapy cannot be predicted reliably, and varies greatly from patient to patient. Some patients have been reported to develop cardiac iron accumulation despite receiving chelation regimens that produce an overall negative systemic iron balance or maintain low body iron levels (36). At present, failure of a specific iron chelator (or combination of chelators) to effectively clear cardiac iron can be recognized only after many months (4) or, clinically, with the abrupt development of cardiac complications. Our observations suggest that measurement of myocardial RR_2 could permit the evaluation of the response of patients to new or altered iron-chelating regimens within weeks. Myocardial RR_2 could also be useful in the rapid evaluation of candidate iron-chelating agents and regimens. Because the proposed method is evidently sensitive to both ferritin and hemosiderin iron (i.e. RR_2 and A , respectively), the effect on both short- and long-term myocardial iron stores could be assessed.

The proposed MR data analysis method, originally introduced by Jensen and Chandra (15), uses the differences in the physical form of ferritin and hemosiderin to separately measure their concentrations. For multi-echo spin-echo sequences, signal decay caused by soluble, dispersed ferritin iron is monoexponential. By contrast, magnetic field inhomogeneities from the insoluble aggregates of hemosiderin iron result in a non-monoexponential signal decay with a strong dependence on ESP. By fitting a previously described model (15) to the signal decay data, two parameters, RR_2 and A , can be determined. The ferritin iron concentration is then calculated from RR_2 , and the hemosiderin concentration is calculated from A . In this study, consistent with the R_2^* difference, the mean absolute difference in A was nonsignificant (data not shown). The proposed RR_2 method has been described theoretically, validated *in vitro* in studies of surrogate phantoms and human liver explants, and

corroborated in investigations *in vivo* of normal control subjects and patients with iron overload (15–17,37). These previous studies were conducted with Carr–Purcell–Meiboom–Gill pulse sequences with relatively long scan times that required respiratory gating *in vivo*. A recent major advance in the applicability of this method has been the development of a breath-hold ME-FSE pulse sequence (18), improving both the accuracy and patient acceptability. In addition, this spin-echo pulse sequence is less sensitive to non-iron-related magnetic field inhomogeneities, which may confound gradient-echo pulse sequences used for the measurement of R_2^* (27).

CONCLUSIONS

Our experimental results show that the new relaxation rate RR_2 can detect decreases of approximately 8% after as little as 1 week of therapy with the oral iron chelator deferasirox. This new CMR method is evidently sensitive to both intracellular ferritin iron and hemosiderin iron (i.e. RR_2 and A , respectively), making possible the assessment of changes in both short- and long-term myocardial iron stores. Increases in myocardial ferritin iron could potentially provide an early warning of a heightened risk of iron-induced toxicity. The measurement of myocardial RR_2 may be a promising investigational method for the rapid evaluation of the response of patients with transfusion-dependent thalassemia to new or altered iron-chelating regimens.

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Abbreviations used

A	aggregation index
BW	bandwidth
CMR	cardiovascular magnetic resonance
ESP	echo spacing
ME-FSE	multi-echo fast spin-echo
R_2	transverse relaxation rate
R_2^*	effective transverse relaxation rate
RF	radiofrequency
ROI	region of interest
RR_2	reduced transverse relaxation rate
T_2^*	effective transverse relaxation time
TE	echo time

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Figure 1.

Representative ME-FSE image sets of a patient acquired with different breath-holds. Left: ESP = 5.6 ms; middle: ESP = 7 ms; right: ESP = 10 ms; top: before registration; bottom: after registration. Note that the third ME-FSE image (third column) was originally shifted with respect to the first ME-FSE image, and that it was adequately co-registered with the other two ME-FSE images after performing the registration algorithm. The dotted lines and arrows were drawn to visually assess the accuracy of image registration.

Figure 2.

Representative log signal *vs* time curves for the three ESPs used in this study, as well as the corresponding signal fits. (a) The individual R_2 values calculated using the monoexponential equation were 20.6, 23.8 and 26.8 ms for ESPs of 5.6, 7 and 10 ms, respectively. (b) The corresponding RR_2 value calculated using the non-monoexponential equation was 17.4 ms. These findings are consistent with the theory of non-monoexponential signal decay in iron overload (15,16).

Figure 3.

Representative pixel-by-pixel RR_2 (left), R_2 (middle) and R_2^* (right) maps of a patient suspending chelation for 1 week (top) and resuming chelation for 1 week (bottom). The RR_2 and R_2 maps are displayed with identical color scales ranging from 0 to 50 s^{-1} , and the R_2^* maps are displayed with color scales ranging from 0 to 150 s^{-1} .

Figure 4.

Bar plots of individual percentage differences in RR_2 (left), R_2 (middle) (using shortest echo spacing) and R_2^* (right) between day 7 and Day 14. Eight of 10 patients showed a decrease in RR_2 , 10 of 10 patients showed a decrease in R_2 and three of 10 patients showed a decrease in R_2^* .

Table 1

Mean myocardial RR_2 , R_2 and R_2^* measurements ($n = 10$). Reported data from observer 1 and analysis 1. Day 7, 1 week after suspending iron chelation; Day 14, 1 week after resuming iron chelation. Both R_2 and RR_2 measurements were significantly different between day 7 and Day 14. By contrast, R_2^* did not change significantly after restarting iron-chelating therapy

Relaxation rate (s^{-1})	Day 7	Day 14	Difference (Day 14 – Day 7)	
			Absolute (s^{-1})	%
RR_2	24.4 ± 4.1	22.7 ± 4.8	-1.7 ± 1.3^a	-7.8 ± 6.2^a
R_2	27.5 ± 5.3	26.0 ± 5.0	-1.5 ± 1.5^b	-5.5 ± 5.2^b
R_2^*	61.4 ± 29.6	61.9 ± 33.4	0.5 ± 11.5	-1.7 ± 27.4

^a
 $p < 0.01$.

^b
 $p < 0.05$.

Table 2

Intra and inter-observer variability in the calculation of myocardial relaxation rates RR_2 , R_2 and R_2^* . The intra- and inter-observer agreements for RR_2 and R_2 calculations were excellent, whereas the corresponding agreements for R_2^* calculation were poorer. Compared with the calculations for the spin-echo-based RR_2 and R_2 , the calculation for the gradient-echo-based R_2^* was more sensitive to the manual segmentation of left ventricular contours. These results suggest that RR_2 and R_2 calculations are highly repeatable and reproducible

Relaxation rate	Intra-observer (Bland-Altman)			Inter-observer (Bland-Altman)		
	Difference (s^{-1})	Upper 95% limit (s^{-1})	Lower 95% limit (s^{-1})	Difference (s^{-1})	Upper 95% limit (s^{-1})	Lower 95% limit (s^{-1})
RR_2	0.03	2.20	-2.14	-0.03	3.29	-3.36
R_2	-0.26	1.42	-1.93	0.45	3.39	-2.49
R_2^*	5.11	25.2	-14.98	5.47	20.20	-9.26